BIOTECHNICAL SYSTEM OF AUTOMATIC CONTROL OF PHOTOSYNTHESIS

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Present achievements and future prospects in the field of cybernetics are such that the problem of optimal control of living systems can now be placed on the agenda for active consideration. This problem assumes its most concrete form in the field of space biology in connection with human life support during long-duration space flights. /460

There is thus an urgent need for creation of a system of optimal control of living organisms in a confined space and volume, i.e., creation of a closed ecological system on the basis of the inclusion of unicellular algae, zooplankton, higher plants, animals and man as its components. This problem is most intimately connected with aspects of biocybernetics. Under natural conditions all plants are subjected to the action of 24-hour rhythms of alternating light and darkness. In response to these rhythms, similar rhythms of physiological activity have arisen in many plants. For instance, the effect of a rhythm of light and darkness on excretion of CO_2 by separate leaves has been demonstrated [10].

Preservation of rhythms in tissues of plants not subjected to periodic stimulation in the form of changes in intensity of light and temperature suggests that a clock mechanism or generator is present in the tissue itself. It is not yet clear whether these cell oscillators should be sought among individual physiological processes or whether such properties are possessed only by whole cells [10]. The external environment, the "external controlling system" directs the work of endogenous "biological clocks," and synchronization of internal and external periodic processes is not disturbed. By the creation of artificial external conditions these rhythms can be disturbed in plants for long periods of time. /461

If external conditions are placed under the control of the plant, the function of a "biological clock" can be developed to a high degree by creating a strong positive link between the control system and the external "controlled system."

If constancy of optimal parameters of the external environment is maintained for a plant organism, the function of the "biological clock" loses its evolutionary significance.

It must be assumed that the plant will become adapted to such a state of the environment if, in principle, it can exist and develop without periodic changes in the external environment.

It is clearly of great interest to undertake prolonged investigations of plants from the evolutionary aspect under two conditions unusual for the organism: when the external environmental parameters are strictly constant and when they are controlled by the plant's own "controlling system." The first investigations of

this type using methods of automation and cybernetics were initiated by V. G. Karmanov and collaborators [3-5]. In these experiments a higher plant switched the light on and off without human assistance entirely in accordance with its own requirements associated with the diurnal course of transpiration.

For a bionic system of control of the source of light of this type, in principle any sensors of the intensity of photosynthesis may be used. If the electronic current at the submolecular level is a universal method of control of metabolism [9], this method is still more applicable in a biotechnical system for the control of the external parameters of the environment by a plant organism. In particular, it is rational to use an amperometric method of measuring the intensity of liberation of photosynthetic oxygen in a system for controlling the period of light and darkness by suspensions of the alga Chlorella.

The literature on this method of measurement is adequate, and the main problem in the recording technique which is discussed is the choice of measuring electrodes [11].

Measurement of the volt-ampere characteristics of individual elements in electrolytes ensures, on the one hand, adequate accuracy of measurement, and, on the other hand, it enables the mobility of certain ions to be distinguished if particular voltages are applied to the electrodes, excluding the remaining ions from the process. /462

Platinum is used as the oxygen cathode for electrochemical measurement of the oxygen concentration in the liquid medium.

It was shown that if the cathode potential was between 0.6 and 0.9 V, the current in the measuring circuit was proportional to the oxygen concentration in solution [1, 12, 13]. It must be emphasized that as yet we do not completely understand electrode processes during the cathodic reduction of oxygen. However, the fact is certain that the concentration of dissolved oxygen is proportional to the current, which is negligibly small in the absence of oxygen. A detailed analysis and description of various types of oxygen cathodes of open type, oscillating and rotating cathodes, and cathodes of closed design is given in the literature [11, 13, 15, 16]. It has been shown that the smaller the electrode, the shorter the delay before it reacts to a change in oxygen concentration [13].

The Soviet literature contains a number of papers on measurement of oxygen concentration in a liquid medium in connection with biological research [2, 6-8]. In particular, in one investigation [8] an amperometric method was used to study kinetics of photosynthesis and respiration of aquatic plants on the basis of changes in the oxygen concentration in the suspension.

The advantage of the amperometric method over the polarographic method is obvious on comparing the sensitivity of measurement (10^{-9} mole/liter oxygen compared with 10^{-7}) and the speed of the electrode reaction to a change in O_2 concentration in the medium (fractions of a second compared with minutes).

To determine the intensity of photosynthesis in relative units, a platinum anode can also be used if the current in the circuit is generated by an external source.

To investigate the photoelectric characteristics of a Chlorella culture we constructed a series of measuring vessels from organic glass.

The platinum measuring electrodes inside the vessel are shown diagrammatically in Fig. 1 (lower diagram). The Chlorella suspension can flow between two cylindrical vessels through a narrow channel 20 mm long, 2 mm wide and 0.2 mm high. The floor of this channel is covered by a platinum plate measuring 12 × 2 mm. This platinum plate acts as recording cathode electrode of the photoelectrochemical process of photosynthesis by cells of the Chlorella suspension.

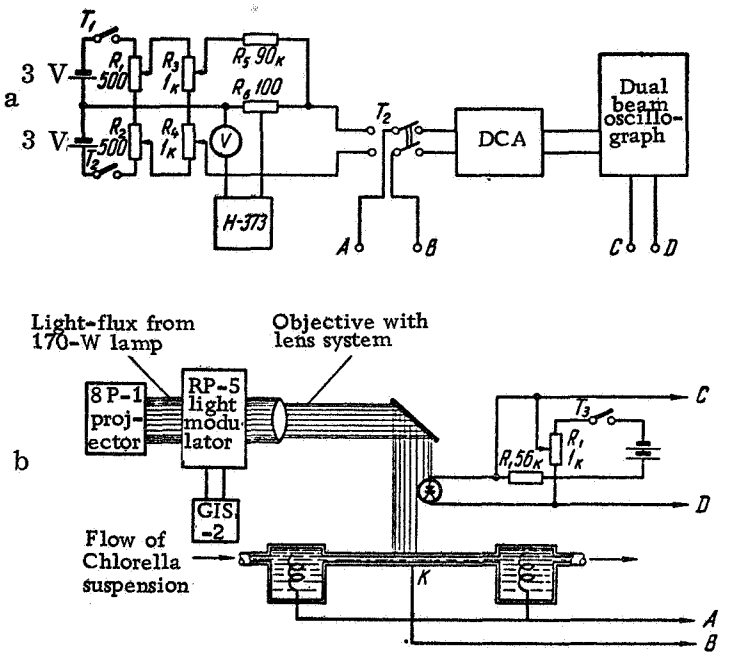

Figure 1. Diagram of System of Measurement of Intensity of Liberation of Photosynthetic Oxygen in Suspension of Chlorella Culture (a) and Construction of Electrode Measuring Cell for Automatic Impulse Control of Light Source and Fixation of Photic Impulses by Photodiode (b).

The organic glass lid, about 0.08 mm in thickness, is hermetically glued to the side walls of the canal. The second recording electrodes consist of two platinum coils wound around a vertical axis in two cylindrical vessels with an internal diameter of 15 mm and height 15 mm. To create a current of the Chlorella suspension through the measuring electrodes and to enrich the suspension with oxygen and carbon dioxide, the two lateral cylindrical vessels are connected to two cultivators with a capacity of 250 ml each, placed at different levels. /463

Because of the glass edge of the cultivators, the volume of Chlorella suspension flowing in unit time can be regulated. A motion picture projector 8P-1 with 170 W incandescent lamp K-30 was used as source of light. The beam of light is interrupted in the narrow focus aperture of the projector by a tungsten flag measuring 2 × 3 mm fixed to the armature of a polarized relay RP-5. Signals from a type GIS-2 dual pulse generator or from the automatic control system depending on the index of intensity of photosynthesis can be applied to the winding of the relay to modulate the light flux.

The signal of the photoelectrochemical process of photosynthesis generated between the active cathode and the two anodes is fed into the measuring circuit (Fig. 1, top diagram). By means of commutation the signals to be investigated can be recorded by means of a dc amplifier (DCA) and dual-beam oscillograph /464
or by means of an automatic writer of N-373 type on squared paper tape. The second channel of the oscillograph is used to record the time of application and interruption of the light flux by means of a type FD-3 photodiode. The equivalent electrical circuit of the system for measuring the intensity of photosynthesis is given in Fig. 2.

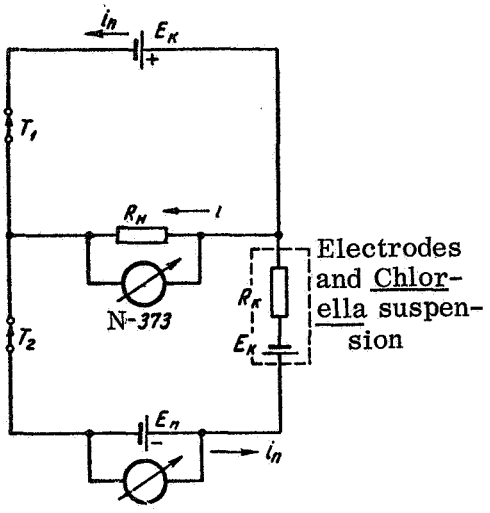

Figure 2. Equivalent Electrical Circuit of Apparatus for Measuring Intensity of Liberation of Photosynthetic O_2 by an Amperometric Method in a Suspension of Chlorella Culture.

The combined current of two parallel circuits produced by the EMF E_n, E_k and E_x flows through the load resistance R_H. When E_n lies between -0.6 and -0.8, E_k represents the EMF determining the initial zero position of the pointer of the automatic writer N-373 in the absence of light flux; E_x represents the EMF on the measuring electrodes.

The fall in voltage at the resistance R_H is proportional to the current in the electrode circuit and determines the intensity of liberation of photosynthetic oxygen.

To feed the signal from the circuit for measuring the intensity of photosynthesis into the automatic control system, a differential circuit and design of the "light" and "darkness" signal detector were used in the executive organ. As signal detectors, two type FD-1 photodiodes were used, fixed on sliding contacts along an arc behind the scale of the N-373 automatic writer and inside the instrument. The photodiodes can be moved along the arc to any scale division to the right or left with its own pointer. This movement can be carried out from the scale side without opening the instrument.

All along the axis of the moveable frame of the instrument, above the horizontal plane of the pointer is fixed a thin optical mirror, used to reflect the

beam of light through 90°, to run parallel to the line of movement of the instrument pointer until it meets the photodiode.

Depending on the direction of the current in the differential circuit of the photodiodes, the polarized relay RP-5 functions in accordance with the conventional signal "light" or "darkness." This signal is applied to the executive element, a second relay RP-5, fixed to the 8P-1 motion picture projector, where its tungsten flag on the armature obstructs or transmits the light flux to the Chlorella suspension. The whole circuit of the automatic system is supplied from a 6-V battery. /465

In this way, our modified N-373 instrument, with a feedback between the Chlorella suspension and the source of light through the circuit of an amperometric system for measuring the intensity of photosynthesis, enables the light—darkness regime of the Chlorella suspension itself to be controlled automatically and at the same time enables this process to be recorded.

Clearly, if all parameters are constant, the rate of change of the intensity of photosynthesis in relative units will be determined by the sensitivity of the apparatus, i.e., by the limit of the measurement scale of the N-373 automatic writer. Consequently, by choosing the measurement scale, the recording can be made at stipulated time intervals on squared paper, to correspond to the reaching of saturation of the photosynthetic process for a stipulated volume of the colony of Chlorella culture. In this position of the automatic writer pointer, the differential circuit may operate and send a signal to the executive organ with the conventional action signal "darkness." After a definite time interval determined by the reaction of the Chlorella suspension to darkness, the curve of the recording changes to the opposite direction. The Chlorella suspension in darkness gradually modifies its function and the liberated photosynthetic oxygen is absorbed in the process of respiration. Movement of the automatic writer pointer is now determined entirely by the process of respiration of the Chlorella culture on the corresponding measurement scale of the instrument.

When it reaches the sixth position of the second photodiode, the beam of light generates a current in its circuit which operates the relay RP-5 into the conventional position of "light" and the Chlorella suspension, after a period of rest, can resume its photosynthetic function.

Having set the limit of measurement and the position of the photodiode on the automatic writer scale, on the basis of the initial velocity of photosynthesis corresponding to a definite time interval, we can bestow on the photoelectrochemical process of photosynthesis of the Chlorella culture the ability to control its own speed and, consequently, to control the times of its light and dark periods, by means of the feedback introduced between the output parameter of photosynthesis and the light flux.

This type of automatic control can be assumed to be optimal if all the other known parameters are optimal (intensity of light flux, temperature, composition of gas mixture, concentration of Chlorella suspension and composition of nutrient medium). /467

Our investigations of a suspension of a culture of Chlorella pyrenoidosa 82 for a period of 6 months in a system providing automatic regulation of periods of light and darkness gave the following results.

1. For a particular volume of Chlorella culture in a particular time interval, with a particular intensity of light flux, temperature, composition of gas medium and composition of liquid nutrient medium, the feedback to the source of light reveals the required rhythm of the light and darkness regime from tracings made by the automatic writer of the intensity of photosynthesis.

2. As well as the natural rhythm of the light—darkness cycle, arrhythmia is also found, when with the onset of darkness or illumination the current in the circuit controlling the intensity of photosynthesis and the polarity of the current do not change and do not obey the external factor, but oscillate around the same level with a period of the order of 1-1.5/min, producing a drop in voltage on the load resistance (1000 Ω) of the order of 0.04 mV. The duration of the period of arrhythmia did not exceed 25 min.

Such a recording of the process investigated is illustrated in Figs. 3, a, b.

This anomalous effect cannot be ascribed to error in the working either of the automatic recording system or of the system of controlling the photoelectrochemical process of photosynthesis. On the assumption that the automatic circuit did not operate and the light flux was not interrupted, the process of increase of current must continue, because saturation of photosynthesis had not yet occurred as subsequent recordings showed.

After a temporary disturbance of the relationship between the changes in electrochemical current and the light and darkness factor of this type, the normal relationship can be restored automatically to its previous form.

It is clear from Fig. 3, c that the photoelectrochemical process in light does not reach our assigned level at which operation of the automatic change from light to darkness must take place. Chlorella suspension apparently holds off or delays the onset of periodic darkening, changes of rhythm of the process, stretching the curve recorded into an S-shape, thereby lengthening the period from 6 to 10 min.

3. Attention is drawn to the reaction time of the electrochemical current passing through the Chlorella suspension in darkness ($T_{r.d.}$) and in light ($T_{r.l.}$).

High photosynthetic function of the Chlorella suspension corresponds to the condition that $T_{r.d.}$ is greater than $T_{r.l.}$ A sign of the rapid impending onset of saturation of the photoelectrochemical process of photosynthesis is a considerable increase in the time $T_{r.l.}$ compared with $T_{r.d.}$. 468

In addition, the parameter itself, the time $T_{r.d.}$ decreases and this may also indicate the beginning of the process of saturation.

There is a direct relationship between the parameter $T_{r.d.}$ and the intensity of light flux and an invert relationship between the parameter $T_{r.l.}$ and the intensity of light flux.

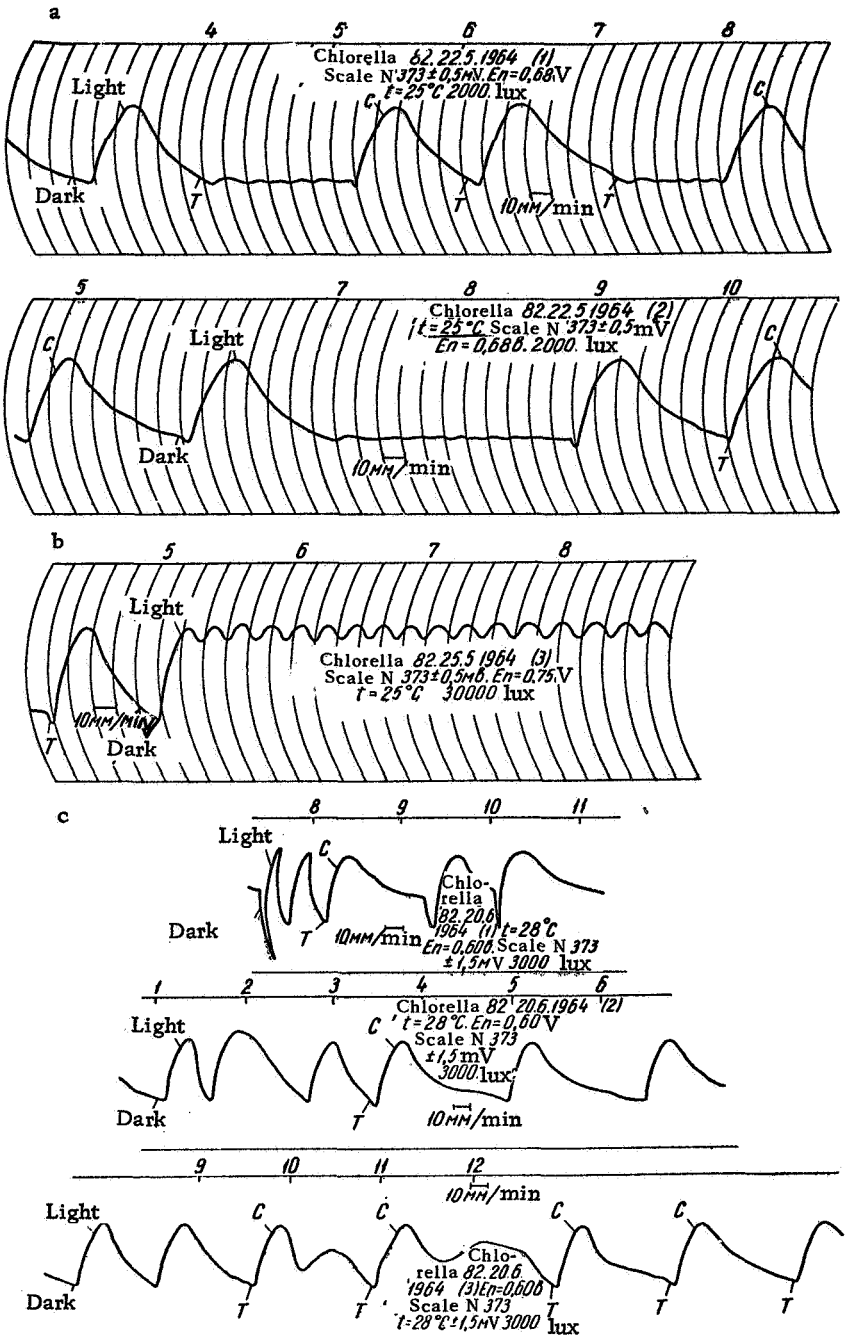

Figure 3. Anomalous Recording of Curve of Current of Photoelectrochemical Process: a and b) After Period of Darkness or Illumination of the Chlorella Suspension, the Polarity of the Current is Unchanged and Periodic Changes Take Place About the Same Level; c) The Current in Response to Light Does not Reach the Previous Value at Which Automatic Switching of the Light Modulator into the "Dark" Position Took Place.

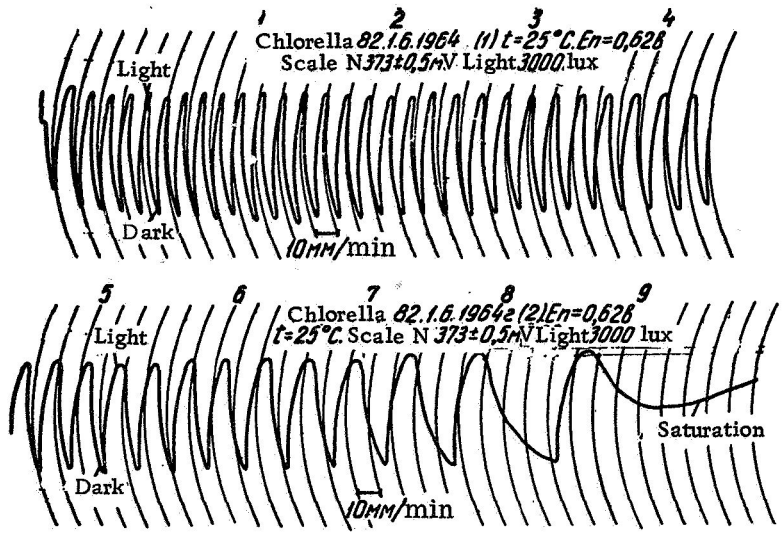

Figure 4. Recording of Photoelectrochemical Current in a Suspension of Chlorella Corresponding to the Process of Saturation of Photosynthesis Taking Place in a Periodic Cycle "Light—Darkness."

Figure 4 shows the beginning of a process for which only 0.3 min was required for the period light—darkness, while at the end of the recording this time is increased to 4 min and saturation of the photoelectrochemical process has taken place.

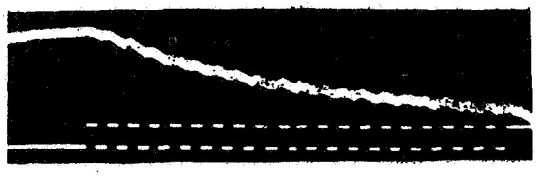

Figure 5. Stepwise Increase in Photoelectrochemical Current in Chlorella Suspension During Illumination with Pulses of Light of Frequency 2 Hz and Duration 0.4 sec.

As a result of electron-oscillographic investigations we were able to determine by an inertialess technique the reaction time of the electrochemical current in darkness ($T_{r.d.}$) and in light ($T_{r.l.}$) using manual control of the duration of the period of light and darkness.

Depending on the duration of the period of light and darkness, delay of the change in the current in the Chlorella suspension varied from 0.4 to 2 sec. With short pulses of light of frequency 2/sec and duration 0.4 sec, the reaction time is considerably shortened and the amplitude of the current greatly reduced. /469

This is in agreement with the fact that under these circumstances the photic energy adsorbed by the suspension falls considerably. The response of the photoelectrochemical current of the photosynthetic process in the Chlorella suspension was never observed to exceed 5 Hz when using a light source with a power of 170 W.

During prolonged illumination of the Chlorella suspension with light pulses at a frequency of 2 Hz and duration 0.4 sec (Fig. 5), summation of the action of photic energy on the photoelectrochemical process is observed as a stepwise curve on the oscillograms.

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